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CHAPTER **28**

Pulmonary Toxicity of Cholinesterase Inhibitors

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I. INTRODUCTION

The lungs are a major organ system of entry into the body and a target for the toxic effects of organophosphorus (OP) compounds, potent inhibitors of the enzyme acetylcholinesterase (AChE). In general, AChE inhibitors (AChEIs) were developed for a variety of indications, including military, medical, and insecticide applications. Nerve agents, OP chemicals with remarkable toxic activity, were first developed by Germany prior to World War II. Whereas nerve agents were produced primarily for military deployment, other cholinesterase inhibitors were used for treating conditions such as myasthenia gravis and as pretreatments for nerve agent exposure. As powerful inhibitors of AChE, these compounds exhibit profound toxicity on multiple organ systems. This chapter discusses respiratory and pulmonary toxicity through direct inhalation of AChEIs and indirect effects on all aspects of respiration through systemic toxicity.

OP nerve agents can be disseminated as liquids or aerosols and are toxic by oral, dermal, or inhalational exposure. The lungs are one of the first organs affected following contact with aerosols and vapors. Lung toxicity by AChEIs is due to the following: (1) parasympathetic muscarinic effects leading to increased glandular secretion throughout the respiratory tract and alveoli, (2) bronchoconstriction from contraction of airway smooth muscle, (3) nicotinic effects on respiratory muscles in the thorax and accessory muscles of the neck causing labored breathing and eventually flaccid paralysis, and (4) central effects resulting in a decrease in respiratory drive.

II. HISTORICAL PERSPECTIVE

The modern age of chemical warfare began during the past century with the development of the present-day vesicants and AChEIs (or OP class nerve agents). There are five OP compounds recognized as nerve agents, designated GA, GB, GD, GF, and VX by their North Atlantic Treaty Organization military abbreviation. The "G" series are named for the fact that they originated in Germany. The A through F designation was based on their chronological order of synthesis. The first nerve agent to be synthesized was tabun (GA) in 1936 by Gerhard Schrader, a chemist at I. G. Farbenindustrie interested in developing OP compounds as insecticides (Harris and Paxman, 1982). This was followed by sarin (GB), named after the four scientists involved in its development (Schrader, Ambrose, Rudriger, and van der Linde) (Harris and Paxman, 1982; Sidell, 1997). The third nerve agent, soman, was synthesized by Richard Kuhn in Germany in 1944 and was termed GD rather than GC since the latter acronym had already been established in the medical literature. Cyclosarin (GF) was the fourth to be synthesized, but interest in this nerve agent declined in favor of the other OP compounds. The fifth agent (VX) was named for being venomous and was synthesized at Porton Down, England, in 1952.

Due to their high toxicity in mammals and volatile nature, some of these fluoride (sarin and soman) and nitrile (tabun) containing OP compounds were further tested, manufactured, and stockpiled by the German military during World War II, but they were never deployed. Some experts believe that Hitler, a victim of a chlorine gas attack during World War I, disliked poison gas and would only use these agents as a last resort. Others speculate that the German High Command mistakenly believed the Allies had developed the nerve agents simultaneously and feared Allied retaliation as the Axis retreated. Nevertheless, tons of nerve agents in munitions were synthesized and stockpiled in Germany during World War II that the United States and Great Britain were not aware of at the time. German tabun production facilities, able to synthesize 100 tons a month, were in place near the end of the war (Saunders, 1957). The former Soviet Union captured an entire nerve agent production facility late in the war and moved it back to Russia, where it started to manufacture and stockpile these agents (Robinson, 1971). Allied forces found that the AChEI nerve agents were 15- to 100-fold more potent than the chemical agents used in World War I.

Since OPs were not used in World War II, the majority of cases of OP toxicity have come from accidental exposure in laboratories and agricultural exposure to OP insecticides. The earliest reported incident of OP toxicity from inhalation came from the laboratory of Willy Lange at the Friedrich-Wilhelms-University. In the early 1930s, Lange and his student, Gerde von Krueger, prepared dialkyl monofluorophosphates and noted their toxic fumes (Holmstedt, 1963; Sidell, 1997). They described the aromatic vapors as leading to dyspnea and laryngeal edema minutes later, followed by a lucid interval, diplopia, and photophobia. The symptoms of toxicity were noted to last several hours before subsiding. Similar clinical pictures have been reported for the OP compounds DFP (Grob *et al.*, 1947), sarin and tabun (Grob and Harvey, 1953, 1958; Krop and Kunkel, 1954), as well as parathion (DuBois *et al.*, 1949). Detailed clinical signs and symptoms have also been described in case reports of accidental exposures to G agents (tabun, sarin, and soman) (Craig and Cornblath, 1953; Craig and Freeman, 1953; Sidell, 1974) and VX (Freeman *et al.*, 1956; Lubash and Clark, 1960; Sidell, 1967).

III. STRUCTURES OF OPs

The general formula and chemical structures of most OP compounds discussed in this chapter are shown in Table 1 of Chapter 2. The nerve agents tabun, sarin, and soman were the most potent compounds in the class, causing lethality to animals in the submilligram range. Their chemical structures are shown in Fig. 1.

Originally developed for the agricultural industry, they contain either an F or CN substituent group and display toxicity to mammals at doses less than 1 mg/kg (Saunders, 1957; O'Brien, 1960). *O*-ethyl-*S*-(2-diisopropylaminoethyl) methylphosphonothiolate (VX), an agent that is less volatile than the G agents, is the most potent AChEI. The first OP synthesized was Bladan, shown to be tetraethyl pyrophosphate (TEPP). The most studied of the OP compounds is diisopropyl fluorophosphate (DFP), originally synthesized by the British, who found it to be more potent than eserine (Adrian *et al.*, 1947).

The phosphorothioates and phosphorodithioates, shown in Table 1 of Chapter 2, were developed when the nerve agents were found to be too toxic and volatile for use in agriculture. These OP insecticides contain a P-S-alkyl and/or a P=S group in their structure. The best known member of this class is parathion, the most widely used insecticide at one time and responsible for more cases of accidental poisoning and death than any other OP compound. The activation and conversion of this weak AChEI (parathion) to the more active and potent form (paraoxon) was demonstrated to take place in the liver (Diggle and Gage, 1951; Gage, 1953).

IV. RESPIRATORY PHYSIOLOGY

The respiratory system can be viewed as two components acting in tandem to facilitate gas exchange, namely the conducting and respiratory portions. The conducting portion supplies the lungs with warmed air on inhalation and allows gases to escape on exhalation, whereas the respiratory portion provides for actual gas exchange between the air and blood. The conducting portion consists of a

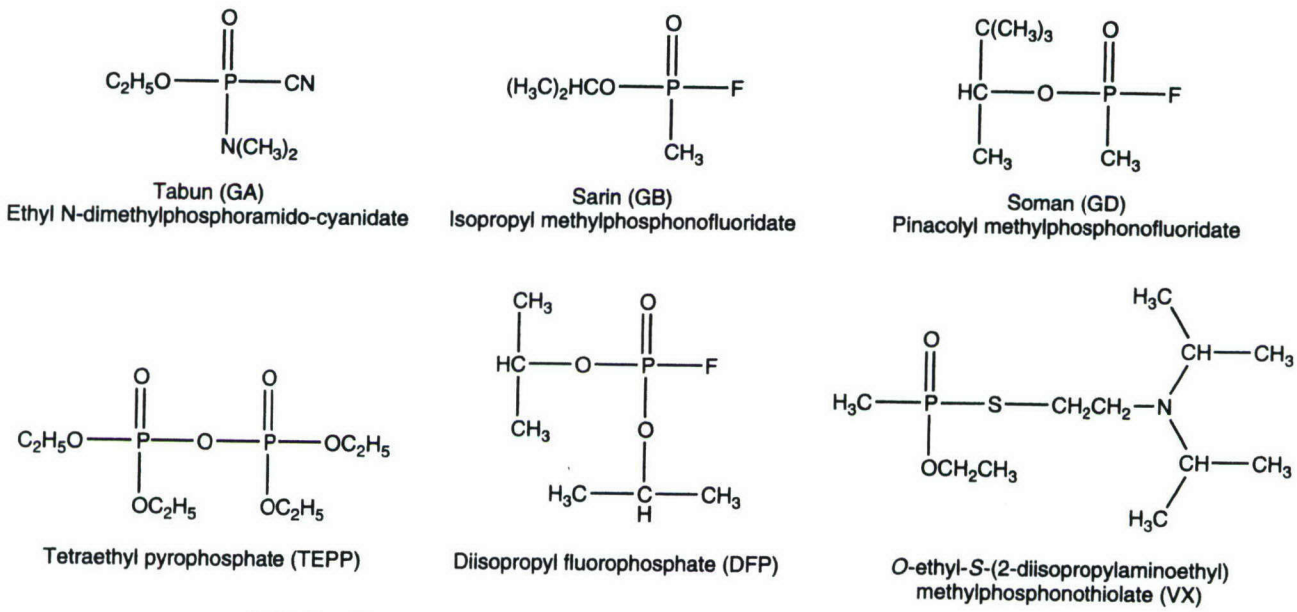

FIG. 1. Chemical structures of OP compounds and their common abbreviations.

branching system of airways, including the nasal cavities, nasopharynx, larynx, trachea, mainstem bronchi, progressing to even smaller bronchi and eventually to bronchioles. This elaborate system of pipes conducts air into and out of the lungs as a result of respiratory movements of the thoracic intercostal musculature and diaphragm. Air reaches the alveolar ducts and finally the alveoli, the site of gas exchange.

The most important factor of respiratory physiology to consider for pulmonary toxicity by AChEIs is the airway resistance through the conducting portion. Airflow through the conducting system from the trachea and mainstem bronchi to the small bronchioles can be characterized as airflow through a series of straight tubes or laminar flow. Jean Poiseuille, a French physician and physiologist, described the volume flow rate through straight circular tubes by the following equation, known as Poiseuille's law (West, 1995; Guyton and Hall, 2005):

$$F = P\pi r^4/8nl$$

where n is the coefficient of viscosity, P is the pressure difference across the length l of the tube, r is the radius of the tube, and F is the volume flow rate. Since the resistance to flow R is driving pressure P divided by flow F using the analogy of Ohm's law, we arrive at the following relationship for flow resistance R.

$$R = 8nl/\pi r^4$$

When applied to airways, if the airway radius decreases by half its original diameter as a result of bronchoconstriction, the airway resistance increases 16-fold. In actuality, the airflow is a mixture of laminar and turbulent flow because the airways must branch to progressively smaller bronchi as they reach the lung periphery. The major site of airway resistance lies in the medium bronchi because the vast number of smaller airways negates any effect they might impose on airway resistance. Airway smooth muscle tone is under the control of the autonomic nervous system. Whereas sympathetic stimulation of adrenergic receptors causes bronchodilation, parasympathetic activity via acetylcholine (ACh) release causes bronchoconstriction. Excess ACh at smooth muscles surrounding airways due to AChE inhibition by OPs produces significant increased airway resistance that is readily characterized on inspiratory and expiratory auscultation of the lungs (West, 1995).

V. CONTROL OF VENTILATION

Since the end result of OP-induced toxicity from lethal doses through inhalation or other routes is asphyxia secondary to respiratory failure, a brief summary of the mechanisms involved in control of ventilation is provided. Although AChEIs affect several aspects of respiration, a detailed review of respiratory physiology and ventilation can be found elsewhere (West, 1995; Guyton and Hall, 2005). The elements of the respiratory control system are the following: chemoreceptors, peripheral sensors, and central sensors, which monitor various measures of respiration to inform the brain; the effector muscles of respiration, which allow for ventilation; and the respiratory control centers in the brain, which integrate the information from the chemoreceptors and regulate the effector muscles.

The lungs contain specialized receptor sensors: the pulmonary stretch receptors, the juxtacapillary or J receptors, and the irritant receptors. The J receptors, found in the alveolar walls close to the capillaries, are very sensitive to chemicals monitored in the pulmonary circulation. Receptor activation in this group leads to rapid, shallow breathing patterns and apneic episodes mediated through the vagus nerve. Furthermore, an increase in the interstitial fluid volume of the alveolar wall will activate these receptors, suggesting a possible role in late stages of OP toxicity after the onset of pulmonary edema. Clinical evidence of an abnormal pattern of breathing (Cheyne–Stokes respiration) has been noted to occur in patients exposed to nerve agents or pesticides. This is an unusual periodic breathing pattern characterized by long periods of apnea interspersed with episodes of hyperventilation (Taylor, 1996). This pattern is due to respiratory insufficiency, hypoxemia, and brain damage and is a grave clinical sign as the tidal volume gradually waxes and wanes prior to death. Direct brain damage from OPs and hypoxemia from respiratory insufficiency secondary to pulmonary congestion and flaccid paralysis of respiratory muscles probably contribute to this respiratory pattern. In addition, the lungs contain irritant receptors located between airway epithelial cells. These groups are activated by noxious fumes, gases, smoke, and dust, inducing a bronchoconstriction reflex that is thought to be responsible for the onset of asthma attacks.

The effector muscles of respiration include diaphragmatic, intercostal, abdominal, and accessory muscles of respiration (e.g., sternocleidomastoid). They are very sensitive to the toxic effects of AChEIs. Accessory muscles of respiration are not a major contributor to the work of breathing under normal conditions, but they come into play during periods of labored breathing. OP compounds are toxic to these muscles of respiration through inhibition of AChE, leading to an excess of ACh, excessive stimulation of nicotinic cholinergic synapses, and eventual flaccid paralysis. Subsequent expansion of the chest wall to inflate the lungs will not occur and respiration will cease.

The respiratory centers are neuronal groups found primarily in the medulla and pons of the brain stem. The medullary respiratory center comprises a dorsal and ventral group, located in the reticular formation of the medulla below the fourth ventricle. They are believed to be central targets for OP toxicity through an unknown mechanism. Damage to these neuronal control centers will affect inspiration and expiration.

VI. EVIDENCE OF PULMONARY TOXICITY

Nerve agents, extremely potent chemicals, are esters of phosphonic acid. A *Ct* [the concentration *C* of agent vapor or aerosol in air (mg/m^3) multiplied by the time *t* of exposure (in minutes)] of 2 or 3 $mg \cdot min/m^3$ of sarin is enough to produce symptoms in man (Johns, 1952). Derived from Haber's rule, the product of *Ct* is a constant such that this *Ct* for sarin can be attained with an exposure to a concentration of 2 or 3 mg/m^3 for 1 min or a concentration of 0.05–0.075 mg/m^3 for 40 min. Only a few milligrams of VX, absorbed through the skin, will cause clinical signs and symptoms of toxicity (Bowers *et al.*, 1964; Craig *et al.*, 1977). The initial signs and symptoms of exposure to small quantities of agent vapor are discussed later. Larger amounts will undoubtedly lead to loss of consciousness, seizure activity, respiratory and cardiac arrest, and death. Clinical effects are evident within minutes of exposure (Ward, 1962), and after a large exposure (*Ct* of 10–200 $mg \cdot min/m^3$), depending on the agent, death is inevitable in 10–15 min without medical intervention. After exposure to a sublethal amount on the skin (1–3 mg), the onset time for clinical effects is typically 1 or 2 hr (Bowers *et al.*, 1964; Craig *et al.*, 1977). The initial effect is usually vomiting, followed by muscular weakness. A lethal amount on the skin (10 mg) in the case of VX, the most toxic by percutaneous absorption, will cause clinical effects within several minutes and death soon thereafter.

A. Clinical Signs and Symptoms

We have learned valuable information regarding the signs and symptoms observed after mild to moderate OP intoxication as it relates to the respiratory system. In most of these clinical cases, the exposure *Ct* is unknown. One study (Craig and Freeman, 1953) analyzed clinical cases of 53 individuals exposed accidentally to tabun (4 cases) or sarin (49 cases). Although miosis and rhinorrhea were the two most consistent signs of toxicity, occurring in 91 and 58% of reported cases, respectively, respiratory symptoms were recorded in 77% of cases of mild to moderate exposure. These cases were considered mild to moderate since symptoms were not severe enough to require atropine therapy, although atropine was administered in several cases.

The types of respiratory symptoms described by patients were not consistent and included coughing, wheezing, increased exertional dyspnea, dyspnea at rest, inability to breathe deeply, and a sensation of pressure in the throat or chest. Rhinorrhea was shown to be accompanied by hyperemia of the nasal mucosa, persisting for the duration of clinical signs and reported symptoms. Since the nose is part of the upper airway, we consider rhinorrhea as part of pulmonary toxicity. Auscultation of the chest revealed prolongation of the expiratory phase and wheezy breath sounds (lung field location unreported) for 5 days following exposure. In addition, the earliest symptom was recorded, and in the majority of cases, chest pressure and rhinorrhea were noted first, taking place 5–20 min after exposure. Miosis and dim vision occurred next, with onset beginning 15–60 min after exposure.

With regard to respiratory symptom severity, exertional dyspnea, wheezing, and cough became more severe over time, with no consistent pattern for regression of respiratory symptoms. Chest tightness, appearing early in the course of intoxication, was the first to disappear, and exertional dyspnea, reported later among cases, persisted the longest among all respiratory symptoms. Furthermore, an earlier onset for respiratory symptoms noted after exposure predicted a more uncomfortable and prolonged clinical course. This was associated with the suspected scenario in which the concentration was high and duration short. In contrast, individuals believed to have experienced more prolonged but lower concentrations of the agent developed symptoms more slowly. Among all cases, there was no correlation between cholinesterase activity and the clinical signs and symptoms, and no differences in symptoms were noted between those exposed to tabun or sarin.

In contrast to GA and GB, VX contains a low vapor tension, resulting in absorption through the skin to produce systemic toxicity rather than inhalation via the respiratory tract. However, there is clinical evidence for pulmonary toxicity after dermal exposure to such agents. For example, case reports of accidental VX poisoning in humans have indicated evidence of respiratory toxicity from liquid agents without copious nasal secretions (Freeman *et al.*, 1956). Since rhinorrhea is a hallmark of inhalational toxicity, this suggests an indirect toxicity of the respiratory system through a systemic route. In addition, respiratory symptoms of chest tightness were late appearing, beginning 24 hr after initial exposure and lasting 3 days, unlike the early respiratory symptoms experienced by patients exposed to the more volatile GA and GB agents (Craig and Cornblath, 1953; Craig and Freeman, 1953). At autopsy, evidence of pulmonary toxicity is quite pronounced, independent of the route of administration. Elevation of the diaphragm and collapse of the lungs are common findings. Further observations indicate pulmonary ischemia and congestion (O'Brien, 1960).

B. Nasal Airway

Since the nasal passages constitute the initial conducting portion of the respiratory tract, we consider toxicity of the nasal mucosa here. The anterior aspect of the nose is the area most prone to toxic effects by any inhalants because it is the initial primary site of deposition of highly volatile vapors and particles (Stott and McKenna, 1984; Dahl and Bechtold, 1985). Rhinorrhea can be an early warning indicator of recent OP exposure because it is very common in case histories after inhalational exposure and is the earliest clinical sign, along with miosis, for the diagnosis of an acute exposure.

Rhinorrhea or "runny nose" occurs because of muscarinic receptor activation from excess ACh secondary to inhibition of AChE. Rhinorrhea usually occurs minutes after an exposure and secretions tend to be thin, clear, and serous in nature. Thick, rubbery secretions may be seen after atropine treatment. Even small amounts of OP vapor can set off a profound rhinorrhea, but the symptoms are typically dose related. One case report demonstrates the abundant rhinorrhea one might experience after exposure to inhaled vapors of sarin. The rhinorrhea was described by the patient to be like a "leaking faucett" (Sidell, 1997). A general increase in secretions from any glands, including nasal mucosa, intestinal, and salivary, can be triggered from dermal or inhalational exposure. From numerous clinical cases, it is known that low *Ct*s will produce a triad of effects on the eyes, nose, and airways. Not only will bronchoconstriction in the airways contribute to dyspnea but also goblet cells and other secretory cells of the nasal mucosa and bronchi will contribute to the dyspnea experienced from OP exposure. Only during moderate exposure will one see deficits in ventilation, copious secretions, and severe dyspnea. Severe exposure will produce cyanosis, loss of consciousness, and convulsions.

C. Trachea and Bronchi

Although the extent of signs and symptoms varies with the type of OP agent, concentration, time of exposure, and route of administration, the predominant signs of exposure include constriction of the airways and increased secretions, leading to various degrees of dyspnea (Taylor, 1996). Similar to rhinorrhea, low levels of exposure to nerve agents such as sarin at a *Ct* of 5–10 mg·min/m^3 will produce respiratory discomfort in the majority of patients, primarily due to bronchoconstriction (Sidell, 1997). The severity of pulmonary complaints will increase as the concentration of the agent or the time of exposure to the agent increases. Although pulmonary function studies have yielded mixed results on the importance of bronchoconstriction in subjects exposed to various low levels of sarin (*Ct*s up to 19.6 mg·min/m^3) (Clements *et al.*, 1952), it is clear that pulmonary changes in airway resistance and tracheobronchial secretions are heard clinically upon auscultation of the lungs (Sidell, 1997). It is important to realize how sensitive lungs are to low levels of nerve agent vapors or aerosols because even a *Ct* exposure of 5 mg·min/m^3 of sarin will produce signs and symptoms of toxicity (Marrs *et al.*, 1996). Patients exposed to sufficient levels of OP compounds that are toxic to the respiratory system will indicate vague symptoms of chest tightness or pressure but show striking pulmonary signs on physical examination. Combinations of wheezing (expiratory, inspiratory, or both), rales, and rhonchi have all been reported in clinical cases. The pulmonary effects begin within seconds after inhalation. If the amount inhaled is large, the patient will exhibit signs of severe dyspnea, poor ventilation, cyanosis, and loss of consciousness.

Although generalized systemic effects are sometimes present depending on the inhaled dose, local effects on the airways are always typically present and are the earliest symptoms recorded following inhalation of nerve agent vapors or aerosols (Craig and Freeman, 1953; Vojvodic, 1981). The airways are particularly vulnerable to the toxicity of AChEIs, considering that inhaled gases will be taken up at a rate of 8–14 breaths/min for an average adult human male. The involuntary smooth muscle that surrounds the airways of the bronchial tree is the target for AChEI activity, leading to bronchoconstriction with subsequent wheezing. Airway smooth muscles contain numerous excitatory cholinergic inputs (Suzuki *et al.*, 1976) and a relative resistance to muscarinic receptor desensitization and muscle fatigue, making them highly vulnerable to AChE inhibition. One study investigating anti-ChE-induced constriction of isolated canine tracheal smooth muscle showed that a very low soman concentrations (10^{-9} *M*) can increase the amplitude and prolong the half-relaxation time of contractions elicited by electric field stimulation (Adler *et al.*, 1992). This was true for all AChEIs examined, including three OP (soman, sarin, and paraoxon) and three carbamate (CM) ChE inhibitors (physostigmine, neostigmine, and pyridostigmine). Contractures of canine airway smooth muscle were detected when AChE activities were reduced by more than 52% (Fig. 2). When contractures are plotted as a function of AChE inhibition for the OPs and CMs, there is a linear rise in contracture amplitudes as AChE inhibition increases. This demonstrates that contracture depends on the degree of AChE inhibition and not on the nature of the inhibitor, suggesting that it is mediated by ACh accumulation. Another experiment compared the ability of two different oximes, pralidoxime (2-PAM) and HI-6, to relax soman- and sarin-induced contractures in canine tracheal smooth muscle (Fig. 3). For nerve agents that undergo rapid aging (soman), 2-PAM was unable to

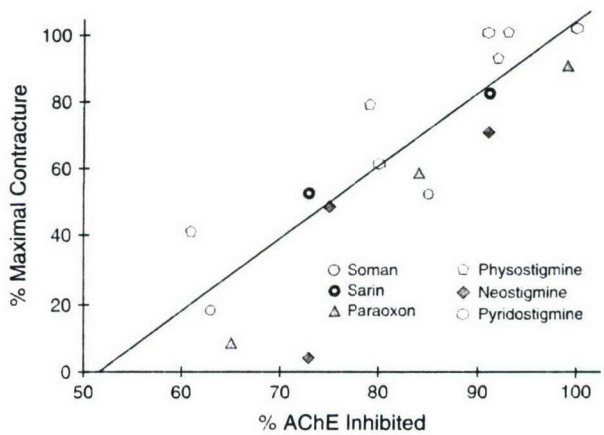

FIG. 2. Contractures plotted as a function of ChE inhibition for three OP and three carbamate AChEIs. Each symbol represents a single muscle strip on which both tension and AChE activity were determined.

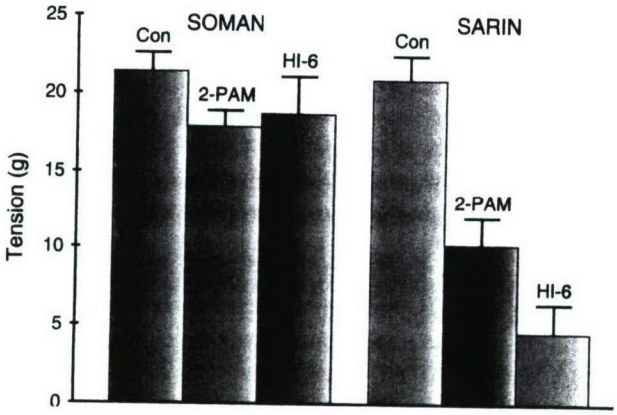

FIG. 3. Relaxation of soman- or sarin-induced contractures in isolated canine tracheal smooth muscle strips by the oximes HI-6 and 2-PAM. Incubation times for HI-6 (100 μM) or 2-PAM (1 mM) were 30 min, and the oximes were added 15 min after soman (0.1 μM) or sarin (0.1 μM) exposure. The symbols represent the mean ± SE of data from four to six muscle strips.

sufficiently reactivate AChE. Surprisingly, the best known AChE reactivator, HI-6, was unable to reactivate AChE inhibited by soman in airway smooth muscle. Both oximes demonstrated an ability to reactivate AChE in the presence of sarin, but HI-6 treatment led to more relaxation of sarin-induced contractures.

D. Bronchioles and Alveolar Cells

One study of sarin effects on rat lungs indicated increased cellular proliferation in the lungs with interstitial thickening 4 days after sarin exposure (Pant *et al.*, 1993). Signs of respiratory bronchiole damage, loss of alveolar spaces, and evidence of lung consolidation occurred 16 days after sarin exposure. A typical combination therapy consisting of atropine, diazepam, and pralidoxime prevented these lung changes. There was significant interest in the toxic effects of trialkylphosphorothioates, contaminants formed during synthesis and storage of P=S phosphorothioate pesticides, because of their ability to produce pulmonary toxicity. Although these are classified as OPs (Clothier *et al.*, 1981), these weak AChEIs do not produce cholinergic changes at doses causing visible lung pathology (Dinsdale, 1992). Trimethyl phosphorodithioate (OSSMeO), a representative of this class, was shown to produce selective type I alveolar pneumocyte damage in rats within 12 hr after oral administration of a lethal dose followed by consolidation of the lungs and alveolar edema (Dinsdale and Verschoyle, 1988). In addition, OSSMeO was shown to cause distortion of rat Clara cells of the bronchiolar epithelium. Similar changes in rat Clara cells, namely hypertrophy, distortion, and cell death, were demonstrated 24 hr after exposure to sublethal doses of OSSMeO (Imamura *et al.*, 1983).

E. Central Respiratory Center

OP compounds affect important respiratory control centers in the brain stem. Although it was known early on that AChEIs cause death by respiratory failure (Modell *et al.*, 1946; Freeman and Himwich, 1949), the majority of the actions were thought to be systemic-related effects on bronchi and respiratory muscles. Central nervous system effects of AChEIs on respiration were not considered a major mechanism until Douglas and DeCandole showed that anticholinesterases caused depression of the respiratory center (DeCandole and Douglas, 1949; Douglas, 1950). The first demonstration of decreased output from the respiratory center was shown by Krivoy and Marrazzi (Krivoy and Marrazzi, 1951; Krivoy *et al.*, 1951). They showed that the output of the respiratory center, recorded as phrenic nerve potentials, was sensitive to the systemic effects of toxic DFP levels. It was also shown that recovery from this central respiratory depression could occur spontaneously or be induced with atropine. Further studies demonstrated similar findings in cats using TEPP (Douglas and Matthews, 1952) and in rabbits, cats, and monkeys using sarin (Holmes, 1952, 1953).

VII. MECHANISM OF RESPIRATORY FAILURE FROM OP TOXICITY

Although it was known that asphyxia from respiratory failure was the cause of death from OP intoxication, it was unclear which component (muscle paralysis, bronchoconstriction, or central respiratory drive) played a greater role. Respiratory failure was shown to be mostly due to failure of the central respiratory drive by Rickett and colleagues (Rickett, 1981; Ricket *et al.*, 1986). They administered 1 LD_{50} of soman, sarin, tabun, or VX every 15 min into a cat until the onset of respiratory arrest. Disruption of the normal firing pattern of the medullary respiratory-related neurons ensued first, followed by changes in phrenic nerve activity, diaphragmatic electromyogram, diaphragm contraction, and airflow. During respiratory arrest, the diaphragm muscle was still able to contract tetanically at 100 Hz for 500 msec, but the medullary respiratory-related units and the phrenic nerve stopped firing.

An early technical report by DeCandole *et al.* (1953) showed similar findings of the importance of a central component in respiratory failure, but this depended on the species and the OP agent. DeCandole *et al.* investigated seven compounds against nine mammalian species and concluded that central respiratory failure predominated, but this depended on the species, the drug used, and the dosage administered. For example, central failure appeared to be the sole cause of respiratory arrest in monkeys. There were also differences between the two studies. Whereas the later study by Rickett *et al.* in cats showed the importance of central respiratory drive, the DeCandole report

hinted at bronchoconstriction as the predominant feature occurring earliest in cats. Another study comparing the effects of bronchoconstriction in dogs and monkeys injected intravenously with sarin (Johnson *et al.*, 1958) supported the earlier findings (DeCandole *et al.*, 1953) of weak bronchoconstriction in monkeys, but canine airway smooth muscle showed significant sensitivity to sarin. In another study involving rabbits exposed to sarin, loss of central respiratory drive and neuromuscular block of diaphragmatic muscles were shown to be responsible for respiratory failure (Wright, 1954). From these studies, it can be concluded that central failure of respiration is most likely the predominant cause of death, which is aided by weakening of respiratory muscles and airway obstruction from increased secretions and bronchospasm. Furthermore, respiratory failure has been shown to precede significant cardiovascular depression (Wright, 1954; Rickett *et al.*, 1986; Sidell, 1997), strengthening the importance of respiratory mechanisms as the primary cause of death.

VIII. THERAPEUTIC STRATEGIES FOR OP INTOXICATION

OP nerve agent toxicity is due to their irreversible inhibition of the enzyme AChE, present at all known cholinergic synapses (Taylor, 1996). AChE limits the duration of the activity of ACh and thus prevents its accumulation at synaptic junctions. Inhibition of AChE results in excessive stimulation of cholinergic synapses, which leads to bronchoconstriction, laryngospasm, muscle weakness, convulsion, and death (Ho and Hoskins, 1987). The standard U.S. military therapy for intoxication by OP compounds consists of administering atropine to antagonize excessive muscarinic stimulation and 2-PAM to reactivate the inhibited AChE. For the nerve agents that undergo rapid aging, such as soman, 2-PAM is inadequate since AChE becomes resistant to reactivation within several minutes of exposure (Berman and Decker, 1986). In this case, the only practical strategy is to protect a critical pool of AChE from irreversible inhibition by pretreatment with the CM pyridostigmine bromide (PB) (Gordon *et al.*, 1978; French *et al.*, 1979). One study showed that when used as a pretreatment against the nerve agent soman, PB could rescue primates from respiratory failure and death (Kluwe *et al.*, 1987). Low doses of PB have been shown to be effective in protecting against soman toxicity when combined with atropine and 2-PAM (Caldwell *et al.*, 1989; Dawson, 1994; Marino *et al.*, 1998). Since pyridostigmine does not enter the blood–brain barrier, peripheral protection of diaphragmatic muscle function and airway musculature is paramount to its mechanism. Although pretreatment with PB followed by treatment with atropine and an oxime represents a rational strategy for protection against soman exposure, newer pretreatment candidates for central protection against OP toxicity would be useful therapeutic adjuncts.

IX. ORGANOPHOSPHATE USE AND ASTHMA

Epidemiological studies have linked OP exposure to wheezing and symptoms related to a hyperactive airway (Deschamps *et al.*, 1994; O'Malley, 1997; Salam, 2004). The establishment of this link is interesting because asthma prevalence has been increasing in the United States. OP insecticide use has increased in agrarian communities (Fenske *et al.*, 2002; Koch *et al.*, 2002) as well as urban populations (Lu *et al.*, 2001; Berkowitz *et al.*, 2003). Therefore, it is not surprising that the largest increase in asthma prevalence has occurred among youth in nonrural populations (Hartert and Peebles, 2000). The proposed mechanism of action to explain OP insecticide effects on asthma has been AChE inhibition, leading to excess ACh, resulting in activation of M3 muscarinic receptors on airway smooth muscle and subsequent bronchoconstriction (Roffel *et al.*, 1990, 1994; Coulson and Fryer, 2003). Studies indicate that the OP insecticide chlorpyrifos induces airway hyperreactivity by a different mechanism in guinea pigs (Fryer *et al.*, 2004; Lein and Fryer, 2005). It was shown that the OP insecticide chlorpyrifos could induce bronchoconstriction in guinea pig airways, and this was independent of AChE inhibition. Autoinhibitory M2 muscarinic receptors on parasympathetic nerves supplying airway smooth muscle prevent airway hyperreactivity (Minette and Barnes, 1988), but chlorpyrifos, parathion, and diazinon inhibit this M2 receptor population.

X. CONCLUSIONS

AChEIs are toxic to multiple organ systems, but the main cause of death is through pulmonary toxicity. OP nerve agents cause toxicity to multiple aspects of breathing, including inhibition of central respiratory drive, constriction of airway smooth muscle leading to bronchospasm and bronchoconstriction, increased airway secretions, and neuromuscular block of diaphragmatic and intercostal muscles. Airway constriction, bronchospasm, and increased airway and nasal secretions compound the dyspnea experienced by the patient, but they can indicate early signs of mild OP exposure. Although death by asphyxiation is predominantly due to central respiratory failure, fatigue and flaccid paralysis of muscles responsible for expanding the chest wall in order to inflate the lungs are contributors to respiratory arrest, leading to hypoxemia, convulsions, brain damage, and death.

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